Lignin + Nootkatone = Dead Ticks

Derived from essential oils of plants such as grapefruit, vetiver grass, and Alaskan yellow cedar, as well as by chemical synthesis methods, nootkatone is commonly used in foods, cosmetics, and pharmaceuticals. But scientists have also demonstrated nootkatone's potential to kill ants, termites, mosquitoes, cockroaches, and ticks, including *Ixodes scapularis* (blacklegged tick), whose bite can transmit bacteria that cause Lyme disease in humans and other animals.

According to the Centers for Disease Control and Prevention (CDC), there were 29,959 confirmed cases of Lyme disease in 2009—the latest year for which statistics are available. Afflicted individuals experience fever, headache, fatigue, and skin rashes. Left untreated, Lyme disease can affect the joints, heart, and nervous system.

For people in regions of the Northeast and Midwest where blacklegged tick populations are endemic, the threat of Lyme disease necessitates sharp-eyed vigilance and a willingness to take preventive measures. These include wearing light clothing to reveal crawling ticks, removing leaf litter where they might hide, creating mulch barriers, spraying insecticides, and using repellents.

KIRBY STAFFORD III (D2049-2)

Nootkatone application in Connecticut by Craig Boland, owner of Grassman, LLC, a company contracted to apply the substance to the perimeters of homeowners' yards. Most ticks are found in the area where the forest meets the yard.

KIRBY STAFFORD III (D2049-1)

Spray residue of nootkatone on leaves.

Biobased Pesticide

For some folks, though, concerns about environmental or personal exposure to chemicals make spraying a measure of last resort. Interest in alternatives has prompted research on natural tick controls.

Although low doses of nootkatone have proven effective against different tick species in the laboratory, the essential oil rapidly turns to vapor when applied in the field. And while nootkatone is environmentally benign and nontoxic to humans, early formulations caused discoloration or other signs of toxicity in plants.

Now, however, a solution to both of these problems could be at hand.

As part of a 3-year cooperative project awarded by the CDC, entomologists Kirby Stafford and Robert Behle are testing a spray-dry procedure that encapsulates nootkatone in lignin. In nature, lignin serves as a kind of molecular mortar that holds together the cell walls of plants. In this case, the researchers used lignin as a semipermeable packaging in which to extend nootkatone's residual activity and slow its environmental loss.

The lignin-encapsulation technology was originally developed and patented for use with other pest-control agents. Investigations by Behle and colleagues at ARS's Crop Bioprotection Research Unit in Peoria, Illinois, determined that the technology could similarly protect nootkatone, improving its effectiveness as a tick control.

Targeting Tiny Tick Nymphs

Stafford, who is with the Connecticut Agricultural Experiment Station (CAES) in New Haven, first began field testing nootkatone in 2008 and later sought Behle's formulation expertise when it became apparent the essential oil lacked sufficient residual activity to kill host-seeking nymphs and those hiding in leaf litter.

"We found that when you apply nootkatone, it doesn't last more than 3 days in the field before control breaks down," says Stafford. This can leave too little time for the oil to move down into leaf litter, where nymphs that aren't seeking hosts like to hide, adds Stafford, who conducted the outdoor trials with CAES postdoctoral researcher Anuja Bharadwaj.

Controlling nymphs is critical because their small size allows them to evade detection long enough to transmit *Borrelia burgdorferi,* the spirochete bacterium that causes Lyme disease. According to Stafford, about 90 percent of all cases of Lyme disease can be attributed to feeding by nymphs, which are most active from late May through July.

Protecting Plants, Too

In greenhouse experiments at Peoria, Behle worked with visiting scientist Lina Flor-Weiler and others to evaluate lignin-encapsulated (LE) nootkatone and compare it to emulsified concentrates (EC), a formulation previously used by other groups to apply the oil under field conditions.

For plant-toxicity tests, the researchers sprayed the leaves of 3-week-old cabbage plants, 7-day-old oat plants, and microscope slide covers with either EC or LE nootkatone at five concentrations. These were based on field application rates of 1.6 grams per meter square. After 2 days, small circular areas where the leaves had been treated were cut, weighed, and visually inspected.

The researchers observed that EC-treated areas generally weighed less and showed more damage than those treated with LE nootkatone, indicating the latter formulation's reduced toxicity to plants. Tests for residual activity on slide covers, used as controls for comparison, showed that 95 percent of the EC nootkatone had disappeared by 5 days versus 45 percent for the LE mixture, indicating it had substantially slowed the oil's volatility.

In tests for lethality to ticks—which included *I. scapularis* and three other species—the insides of small glass vials were coated with five different concentrations of nootkatone. In each vial, the researchers placed 10 unfed nymphs and recorded their survival 24 hours after exposure to the treatments.

Each of the four tick species (*I. scapularis, Amblyomma americanum, Dermacentor variabilis,* and *Rhipicephalus sanguineus)* succumbed to nootkatone. But the lone star tick, *A. americanum,* required a slightly higher dose than the others, possibly due to its larger size.

"This is the first report that directly compares toxicity of nootkatone to four target species of tick," the ARS-CAES team notes in a paper accepted for publication in the *Journal of Economic Entomology*.

Home Defense

In 2009, trials conducted on residential properties in Connecticut also showed promise. For those tests, Stafford and Bharadwaj sprayed LE nootkatone along the perimeter of the homeowners' properties where the yards met the forest, with the treatment area extending 3 feet into the yards and 3 feet into the bordering forest. This buffer zone, says Stafford, "is where 82 percent of ticks are normally found."

The trial, which included nontreated homes as controls, ran from June through July. Although the researchers were not able to detect surface residues of the LE nootkatone, they did detect traces of the oil that had found its way below the leaf litter. They observed no signs of burning or other plant damage. Most importantly, "We did not recover any live ticks from the treated sites for the rest of the summer," says Stafford, who adds that final field efficacy data are pending.

In June 2010, they began a third round of tests, treating nine total residential properties—five of them using another nootkatone-encapsulating formulation Behle devised.

Stafford ventures that if the costs of obtaining nootkatone can be reduced, the essential oil could be especially attractive to a burgeoning organic lawn care movement in the Northeast. Rather than a stand-alone defense, however, nootkatone would be integrated with other measures as part of a "biorational" approach to insect control.—By **Jan Suszkiw,** ARS.

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A nymph-stage blacklegged tick on a leaf. Infected nymphs transmit the pathogen that causes Lyme disease. About 90 percent of all cases of Lyme disease can be attributed to nymph feeding.